STABILITY OF SELF-INCOMPATIBILITY AND CROSS-COMPATIBILITY IN PARTIALLY SELF-INCOMPATIBLE ALFALFA CLONES

T. A. CAMPBELL and G. R. BAUCHAN

Plant Sciences Institute, Beltsville Agricultural Research Center, Agricultural Research Service 20705, U.S.A. Received 21 Sept. 1989, accepted 15 Feb. 1990.

The stability of self-incompatibility and cross-compatibility in 10 partially self-incompatible clones selected as potential parents for hybrid seed production was evaluated in the growth chamber. Clones (nulliplex for a completely dominant "red root" (RR) trait) were cultured in four regimes (allowed to develop at a constant 22°C or 27°C or allowed to develop at 22°C then acclimated at 27 or 35°C for 72 h) then self-pollinated or crossed with one of two clones that were quadraplex or triplex for the RR trait. Clones transferred to 27 or 35°C were returned to 22°C after 1 wk. Seeds/flower tripped (S/FT) were computed and data derived from cross-pollination were adjusted using the formula S/FT × (% RR seedlings/100) before analysis. Mean S/FT were 0.81, 0.88, 0.66, and 0.36 for the 22°C, 27°C, 22°C/27°C and 22°C/35°C regimes, respectively. Temperature regimes had no significant effect on self-incompatibility but did affect cross-compatibility significantly in some clones. There was more autogamy at 27°C and in the 22°C/27°C regime than in the other regimes. Mean percent RR seedlings was 78.3 and clones did not differ significantly in percent hybrid seed production. Variation in autogamy observed in these clones was not correlated significantly with variation in hybridity. Stability analyses revealed considerable variation among the clones in terms of potential for hybrid seed production.

Key words: *Medicago sativa* L., autogamy, allogamy, red root, stability, hybrid, heterosis, heat shock

[Stabilité de l'auto-incompatibilité et de la compatibilité croisée dans les clones de luzerne partiellement auto-incompatibles.]

Titre abrégé: Stabilité de l'auto-incompatibilité et de la compatibilité croisée chez la luzerne.

Nous avons évalué la stabilité, en chambre de croissance, de l'auto-incompatibilité et de la compatibilité croisée de 10 clones partiellement auto-incompatibles sélectionnés comme parents potentiels pour la production de semences hybrides. Des clones [nulliplex pour un caractère "racine rouge" (RR) entièrement dominant] ont été mis en culture et exposés à quatre différents régimes de température (développement à une température constante de 22 ou 27°C ou développement à une température de 22°C puis acclimatation à une température de 27 ou 35°C pendant 72 h); ils ont ensuite été fécondés par autogamie ou par croisement avec un deux des clones quadraplex ou triplex pour le caractère RR. Les clones exposés à une température de 27 ou 35°C ont été ramenés à une température de 22°C après une semaine. Le nombre de semences/fleur (S/FT) a été noté et les données sur la pollinisation croisée ont été corrigées par la formule S/FT × (% plants RR/100) avant analyse. Les valeurs moyennes de S/FT pour les traitements à 22°C, 27°C, 22/27°C et 22/35°C ont été respectivement de 0,81, 0,88, 0,66 et 0,36. Les différents régimes de température n'ont eu aucun effet significatif sur l'auto-incompatibiliteé, mais ils ont influé de façon significative sur la compatibilité

croisée de certains clones. Par ailleurs, l'autogamie a été plus marquée à 27°C ainsi qu'à 22/27°C qu'avec les autres régimes. Le pourcentage moyen de plants RR a été de 78,3%, les clones n'affichant aucune différence significative quant au pourcentage de semences hybrides. Nous n'avons observé aucune corrélation significative entre la variation du taux d'autogamie dans ces clones et la variation de l'hybridité. Enfin, les analyses de stabilité indiquent aux taux élevé de variation entre les cones, pour ce qui est du potentiel de production de semences hybrides.

Mots clés: *Medicago sativa* L., autogamie, allogamie, racine rouge, stabilité, hybride, hétérosis, choc thermique

Improvements in genetic yields in alfalfa have been relatively small in comparison to those realized in most grain crops (Hill et al. 1988). Total genetic yield improvement in alfalfa between 1956 and 1974 was only 3% and the slow rate of progress can be attributed to several factors including the added complexities associated with breeding for improved yield in a perennial vs. an annual species, diversion of the breeding effort from yield to pest resistance, and poor exploitation of nonadditive gene action (Hill et al. 1988).

High levels of inbreeding depression and evidence of heterosis led to early consideration of the development of hybrid alfalfa (Rumbaugh et al. 1988). Three systems that exploit (1) self-sterility, (2) cytoplasmic male sterility, or (3) female-sterility have been proposed. In 1944, Tysdal and Kiesselbach recommended the production of hybrid alfalfa through the use of highly self-sterile parents. Barnes et al. (1972) concluded that the stability of self-sterility in alfalfa was not sufficient for the commercial production of hybrid seeds without the use of marker genes to verify hybridity and advocated use of a cytoplasmic male sterility (CMS) system. Unfortunately, the production of hybrid seeds using the CMS system has not proved economical because of poor seed production when the male-sterile and pollenizer plants are seeded in separate rows to permit mechanical seed harvesting (Viands et al. 1988). Economical seed production may not be possible until male sterile and pollenizer plants can be randomized in the field with an increased ratio of pollenizers to male-steriles and the use of more efficient pollenizers. Hybrids could be identified during seed conditioning by greater seed size (Pedersen and Barnes 1973) or through marker genes (Hunt et al. 1976). Brown and Bingham (1984) describe a third hybrid seed production system which uses a male- or self-sterile seed parent and a female-sterile pollenizer.

Major advantages to the use of systems 1 or 3 for hybrid alfalfa seed production are that parents could be randomized in the field to improve seed set and that F_1 seeds could be harvested from all plants. Major disadvantages are the necessity for large-scale field establishment of a small number of genotypes and the lack of stability in self-sterile alfalfa clones. We doubt that it would be economically feasible to establish parents as vegetative cuttings, but establishment of somatic embryos mass-produced in a bioreactor may be possible (Stuart et al. 1987; Chen et al. 1987). The most likely delivery system for these embryos is "artificial seeds", each consisting of an embryo surrounded by a membrane-enclosed gel and nutrient matrix (Stuart et al. 1987) or of an encapsulated dehydrated embryo (McKersie et al. 1988).

Because self-incompatibility refers to failure of fertilization and does not include post-fertilization abortion, Viands et al. (1988) recommend the use of the term "self-sterility" to refer to failure of seed production following self-fertilization due to unknown or unspecified causes. Self-incompatibility in higher plants is generally classified as sporophytic if the incompatibility phenotype of the pollen is determined by the genotype of the pollen parent and gametophytic if the genotype of the individual microspore determines the pollen phenotype (de Nettancourt 1977).

de Nettancourt (1977) identifies the genus *Medicago* as being homomorphic, monofactorial (single *S* locus), and gametophytic. Y. He and Wu (1987) concluded that self-incompatibility in the alfalfa cultivars they studied was gametophytic and was conditioned by a single *S*-locus with multiple alleles; they determined that the reaction site was within the ovarian cavity. In their review of self-incompatibility in alfalfa, Viands et al. (1988) concluded that the self-incompatibility reaction occurs between the ovary and pollen tube, but could cite only unsuccessful attempts to determine the genetic basis of the phenomenon. Mistinova (1987) determined that nonadditive gene action was most important in the control of compatibility among self-incompatible alfalfa clones; he also concluded that cytoplasmic factors were involved. The highest general combining ability was found among clones that were least stable under environmental change.

Many scientists have reported that temperatures ranging from 32°C to 60°C lead to a breakdown of self-incompatibility (de Nettacourt 1977); the response is usually restricted to a gametophytic system although short-term high temperature exposure is known to repress self-incompatibility in crucifers where the system is generally sporophytic (Okazaki and Hinata 1987). In a 9-yr (1958–1966) field evaluation of five alfalfa clones ranging from 9 to 66% self-fertile, Melton (1970) noted that the self-fertility of certain clones was more stable across environments than that of others. Mean self-fertility of the most self-sterile clone ranged from 21% (1959) to 2% (1961). Straley and Melton (1970) evaluated the effects of four temperatures (16, 21, 27, and 32°C) on the self-fertility of eight alfalfa clones representing a range of self-fertilities. Self-fertility ranged from 1.2 to 79% and was generally greatest at 27°C, however, there was a significant clone × temperature interaction. One clone remained relatively stable across all temperatures, another was most self-fertile at 16°C, while a third was most self-fertile at 32°C. The authors concluded that the clones could be grouped into self-fertility classes that would remain relatively constant across temperatures. In another evaluation of five of the eight clones, Dane and Melton (1973) observed a slight increase in self-compatibility when temperatures increased from 21 to 27°C and a decrease in self-compatibility at 32°C. Again, there was a highly significant clone × temperature interaction, reflecting the fact that variations in temperature had a more pronounced effect on the highly self-compatible clones than on those with intermediate or low self-compatibility. Compared to the lower temperatures, in vitro pollen germination decreased significantly when clones were cultured at 32°C. The authors concluded that differential responses to temperature could complicate the characterization of alfalfa clones for self-compatibility if they were not evaluated in the same environment.

The objective of our study was to evaluate the stability of self-incompatibility and cross-compatibility of 10 partially self-incompatible (SI) clones selected in the greenhouse from two broad-based alfalfa populations (BMP8-AC3 and W10-AC3) as potential parents for hybrid seed production (Elgin and Bauchan 1986).

MATERIALS AND METHODS

Effects of Temperature on Self- and Cross-Compatibility

Experiment 1 assessed the effects of 27°C on self-incompatibility and cross-compatibility. Plants were cultured in Chamber 1 (18-h photoperiod provided by 64, F96T12 cool-white fluorescent tubes and 8, 100 W clear incandescent bulbs; photosynthetic photon flux (PPF) of 200 μmols s^{-1} m^{-2} measured at 0.86 m from the light source); experimental design was a split-plot with three replications. Whole plots were clones and there were two subplots (plants) per whole plot. For each whole plot, five racemes on one plant were self-pollinated by tripping each flower with the tip of a plastic label and five racemes on the other plant were crossed with one of two clones quadraplex or triplex for a completely dominant, completely penetrant "red root" (RR) trait (Barnes and Hanson 1967). Pollen from a particular RR clone was used for an entire replication. All of the SI clones were nulliplex for the RR trait. A mean

of 42.6 ± 14.8 flowers were tripped per experimental unit. Seeds/flower tripped (S/FT) were computed after one month for each experimental unit; data derived from cross-pollination were adjusted using the formula S/FT $\times$ (% RR seedlings/100) before analysis. Because means and variances tended to be proportional, all data were transformed using the formula $\log(x + 1)$. There were some missing cells and least squares analyses were conducted (Statistical Analysis Institute, Inc. 1985).

The effects of three temperature regimes (RE's) on self-incompatibility and cross-compatibility were evaluated in the growth chamber in Exp. 2. Treatments in each regime were arranged in a randomized complete block design with three replications. Experimental units were racemes; one raceme on a clone was self-pollinated using the tip of a plastic label and another crossed with one of the two RR clones. Treatments were arranged within each regime as 10×2 complete factorials. Pollen from a particular RR clone was again used for an entire replication. A mean of 29.5 ± 9.0 flowers were tripped per raceme. Plants in RE 1 were allowed to develop in Chamber 1 at 22°C; those in RE 2 were allowed to develop in Chamber 1 at 22°C, then acclimated at 27°C for 72 h in Chamber 2 (an 18-h photoperiod provided by 20, F96T8 cool-white fluorescent tubes and 8, 100 W clear incandescent bulbs; PPF was 100 μmols s^{-1} m^{-2} measured at 0.86 m from the light source); those in RE 3 were allowed to develop in Chamber 1 at 22°C, then acclimated at 35°C for 72 h in Chamber 2. Clones in RE's 2 and 3 were returned to 22°C (Chamber 1) 1 wk after pollination. Seeds/flower tripped were computed and data were adjusted and transformed as described for exp. 1. Because there were some missing cells, least squares analyses were conducted. For each clone $\times$ (self-pollinated vs. cross-pollinated) combination, the following contrasts were partitioned and tested: RE 1 vs. RE 2 and (RE 1 and RE 2) vs. RE 3.

Evaluation of Hybridity
Progenies (experimental units) from the SI parents for each of the two experiments were evaluated separately in Chamber 1 at 27°C. Seeds were planted in galvanized metal flats (55 cm (length) $\times$ 31.5 cm (width) $\times$ 6.5 cm (depth)) filled to within 2.5 cm of the top with pasteurized potting soil. Scarified seeds (240 flat^{-1}) were planted in rows, 0.5 cm deep; spacing was 3.8 cm between rows and 1.9 cm within rows. Experimental design for each experiment was a randomized complete block with three replications. Progenies from each original replication were kept together in a block; plot size varied depending on seed production. Seedlings were pulled from the soil after 2 wk, rinsed, dipped in 0.5 N HCl to intensify the pigmentation, then evaluated against a white background. Percent RR seedlings was computed for each experimental unit and data were arcsin transformed for analyses of variance. Because there were missing cells, least squares analyses were conducted.

Stability Analyses
Lin and Binns (1988) have developed a simple and effective procedure for selecting superior cultivars based on their general performance over environments. Their Superiority Measure (P_i) is defined as

$$P_i = \sum_{j=1}^{n} (X_{ij} - M_j)^2 / (2n)$$

where:
X_{ij} is the response of the ith clone to the jth environment, M_j is the maximum response to environment j, and n is the number of environments.

General superiority over environments and values of P_i are inversely related. The distribution property of P_i is not known but this parameter can still serve as the basis for an empirical ranking of cultivar responses and provides an excellent means of summarizing the compatibility and hybridity data for the present study.

The procedure was modified for the evaluation of the SI clones. P_i values (where $n=4$) were computed for each clone from mean S/FT values derived from self-pollination (M_j=minimum response) or cross-pollination (M_j=maximum response) and from mean percent RR seedlings (M_j=maximum response).

RESULTS AND DISCUSSION

Effects of Temperature on Self- and Cross-Compatibility
Correlations between autogamous and allogamous seed production were 0.26NS (adjusted for percent hybridity), and 0.29NS (unadjusted) for exp. 1, and 0.10NS (adjusted) and 0.22* (unadjusted) for exp. 2. These results indicate that estimates of allogamous seed production for some of the more self-fertile clones may have been inflated by autogamy, especially in exp. 2, and that the adjustment for hybridity was justified.

Table 1. Mean squares from analyses of variance of the effects of a constant 27°C (exp. 1) or three other temperature regimes (exp.2)† on self-compatibility in 10 partially self-incompatible alfalfa clones and on cross-compatibility with two clones carrying a red root (RR) marker gene

	Compatibility (seeds/flower tripped‡)		Hybridity (% RR seedlings)§	
Source	Constant 27°C	Three temperature regimes	Constant 27°C	Three temperature regimes
Temperature regime (RE)	—	0.29NS	—	0.3NS
Clones	0.18*	0.19NS	1.1NS	1.1NS
Self-pollinated vs. cross-pollinated (S vs. C)	4.10**	8.36**	—	—
RE × clones	—	0.10NS	—	1.2NS
RE × (S vs. C)	—	0.45*	—	—
Clones × (S vs. C)	0.12NS	0.06NS	—	—
RE × clones × (S vs. C)	—	0.11NS	—	—
CV (%)	44.4	87.8	21.4	34.0

†Constant 22°C or allowed to develop at 22°C but pollinated after 72 h at 27°C or 35°C then returned to 22°C after 1 week.
‡Data derived from cross-pollination adjusted using the formula seeds/flower tripped × (% RR seedlings/100) before analysis; all data transformed before analysis using the formula $\log (x + 1)$.
§Arcsine transformed before analysis.
*,**Significant at the 5% and 1% levels, respectively; NS, nonsignificant.

The self-pollinated vs. cross-pollinated (S vs. C) contrast was a highly significant (0.01 level) source of variation in both experiments; however, clonal effects were only significant in exp. 1 (Table 1). For exp. 2, the only other significant source of variation was RE × (S vs. C). Mean S/FT were 0.88, 0.81, 0.66, and 0.36 for exp. 1, RE 1, RE 2, and RE 3, respectively, reflecting perhaps the negative effect of 35°C on pollen germination. Some variation in self-incompatibility is evident in both experiments (Table 2) with, for example, Clone 710 producing 0.02 and 0 S/FT in exps. 1 and 2, respectively, while Clone 740 produced 0.73 and 0.25 S/FT, respectively, in these experiments. Variations in female fertility cannot be separated from variations in compatibility with the RR clones in these experiments; however, some variation in allogamous seed production was also evident with, for example, Clone 71 being more productive than Clone 159. The Clone × (S vs. C) contrasts revealed significant variation only in allogamy and only for clones 710 (RE 1 vs. RE 2, 2.10 vs. 0.42 S/FT), 589 ((RE 1 and RE 2) vs. RE 3, 1.20 vs. 1.70 S/FT) and 598 ((RE 1 and RE 2) vs. RE 3, 1.67 vs. 0 S/FT).

When considering the potential of these clones for the field production of hybrid seeds, it is encouraging to note that the four temperature regimes did not appear to have a marked effect on the self-incompatibility mechanism. There was substantially more variation among the self-incompatible clones reported on in the literature than there was observed in our experiments; however, the clones we evaluated were probably selected more rigorously for stability of reaction than those examined by other investigators. It is interesting to note that there were more seeds produced through autogamy in exp. 1 (0.33 S/FT) and in the 22°C/27°C regime than in RE 1 or RE 3 (Table 3). These results are consistent with the work of Straley and Melton (1970) and Dane and Melton (1973) where mean self-fertility was maximized at 27°C. However, autogamy for RE 2 was not significantly greater than that for RE 1 or RE 3 based on t-tests. Variation in allogamy was not pronounced, but these results do indicate that environmental variation can have an effect on the cross-fertility of individual alfalfa clones and that clones being considered for use in hybrid combination should be evaluated in the field under several environments to assess their stability.

Table 2. Effects of temperature on self-compatibility in 10 partially self-incompatible alfalfa clones and on cross-compatibility with two clones carrying a red root (RR) marker gene

	Compatibility (Seeds/flower tripped)				Hybridity†				Stability‡		
	Constant 27°C§		Mean for three temperature regimes¶		Constant 27°C		Mean for three temperature regimes				
Clone	Self-pollinated	Cross-pollinated//	Self-pollinated	Cross-pollinated	No. seedlings	RR seedlings (%)	No. seedlings	RR seedlings (%)	Self-pollinated P_i††	Cross-pollinated P_i	Hybridity P_i
10	0.52	2.57	0.34	1.14	255	88.5	90	66.5	101.4	1.0	306.3
45	0.21	1.08	0.00	0.57	64	68.8	35	71.7	4.2	2.4	182.9
71	0.47	2.06	0.28	2.16	244	88.7	160	68.4	59.5	0.5	268.8
82	0.10	1.82	0.00	1.58	213	85.9	57	98.5	0.8	1.6	29.3
159	0.13	0.56	0.09	1.09	44	87.4	38	97.9	7.7	2.1	41.2
536	0.52	0.93	0.17	1.36	159	83.9	129	77.1	56.8	1.4	422.9
589	0.47	1.12	0.11	1.25	219	79.1	132	79.7	31.1	0.8	165.0
598	0.14	0.57	0.01	1.44	75	55.7	99	63.6	2.0	1.6	1248.6
710	0.02	2.09	0.00	1.09	210	87.3	95	78.2	0.0	1.2	1043.6
740	0.73	1.50	0.25	1.84	169	66.4	153	79.1	87.7	0.4	296.0

†Based on the percentage of seedlings from crosses with the RR clone that displayed the RR trait.

‡$P_i = \sum_{j=1}^{n} (X_{ij} - M_j)^2 / (2n)$ where X_{ij} is seeds/flower tripped or %RR seedlings for the ith clone in the jth environment, M_j is the minimum (clones self-pollinated) or maximum (clones cross-pollinated) response to environment j, and n is the number of environments.

§Experiment 1.

¶Experiment 2: Constant 22°C or allowed to develop at 22°C but pollinated after 72 h at 27°C or 35°C then returned to 22°C after 1 wk.

//Means adjusted using the formula seeds/flower tripped × (% RR seedlings/100).

†† × 100.

Table 3. Effects of three temperature regimes† on the self- and cross-compatibility‡ of 10 partially self-incompatible alfalfa clones

Pollination	Temperature regimes§		
	22°C	22°C/27°C	22°C/35°C
Self	0.08	0.22	0.07
Cross	1.62	1.21	1.10

†Constant 22°C or allowed to develop at 22°C but pollinated after 72 h at 27°C or 35°C then returned to 22°C after 1 wk.

‡Crossed with two clone selected for a dominant red root (RR) marker gene; means adjusted using the formula seeds/flower tripped × (% RR seedlings/100).

§Means within a regime are not significantly different from each other according to t-tests.

Evaluation of Hybridity

No sources of variation were significant in either experiment (Table 1). Mean percent RR seedlings was 79.7 in exp. 1 and 76.9 in exp. 2. Although clones did not differ significantly in percent hybrid seed production, there was some variation among the clones with, for example, Clones 82 and 159 consistently producing a greater percentage of hybrid seeds than Clones 45, 598, or 740 (Table 2). Consistently high levels of hybridity as well as cross-compatibility will be necessary if the self-incompatibility system for hybrid seed production is to be of practical use.

Correlations between seed production from self-pollination and percent RR seedlings were nonsignificant ($r = 0.08$ and -0.16 for exp. 1 and exp. 2, respectively) indicating that the variation in autogamy observed in these partially self-incompatible clones was not related to variations in hybridity. Thus, when choosing clones as potential parents for hybrid seed production, it may be advisable to not screen too stringently for reduced autogamy and to make the final selections on the basis of cross-fertility with other potential parents, stability of the self-incompatibility reaction in the field, and specific combining ability for forage yield.

Stability Analyses

While several clones exhibited excellent self-incompatibility, female-fertility, or hybridity, no clone was generally superior in all three categories (Table 2). Clone 10, for example, ranked low in self-incompatibility and hybridity, but high in female fertility. Clone 159 exhibited relatively high self-incompatibility and hybridity, but poor female fertility. Clone 710 had relatively low P_i values for self-incompatibility and female fertility, but among the highest value for hybridity. Despite a moderately high P_i value for female-fertility, Clone 82 displayed excellent self-incompatibility and hybridity and would appear to be the best choice of the 10 clones for hybrid seed production.

CONCLUSIONS

Because partially self-incompatible alfalfa clones that produce stable, high levels of hybrid seed can be selected, the self-incompatibility system of hybrid seed production may have potential. Consistent with the literature, autogamy was maximized at 27°C and high temperatures had a deleterious effect on seed set. The variation in autogamy among these clones was not correlated with variations in hybridity indicating that final selection of parents for hybrid seed production should perhaps be on the basis of cross-fertility with other potential parents, stability of self-incompatibility in the field, and specific combining ability for forage yield. The potential of the self-incompatibility system for hybrid seed production will not be realized until self-incompatible parental clones that produce a stable, high percentage of F_1 seed in the field are selected and an economical method for the field establishment of clonal material is developed.

Barnes, D. K. and Hanson, C. H. 1967. An illustrated summary of genetic traits in tetraploid and diploid alfalfa. USDA Technical Bulletin 1370. U.S. Government Printing Office, Washington, D.C.

Barnes, D. K., Bingham, E. T., Axtell, J. D. and Davis, W. H. 1972. The flower, sterility mechanisms, and pollination control. Pages 123–141 in C. H. Hanson, ed. Alfalfa science and technology. Agronomy no. 15. Am. Soc. Agron., Madison, Wis.

Brown, D. E. and Bingham, E. T. 1984. Hybrid alfalfa seed production using a female-sterile pollenizer. Crop Sci. 24: 1207–1208.

Chen, T. H. H., Thompson, B. G. and Gerson, D. F. 1987. In vitro production of alfalfa somatic embryos in fermentation systems. J. Ferment. Technol. 65: 353–357.

Dane, F. and Melton, B. 1973. Effect of temperature on self- and cross-compatibility and in vitro pollen growth characteristics in alfalfa. Crop Sci. 13: 587–591.

de Nettacourt, D. 1977. Incompatibility in angiosperms. Springer-Verlag, New York, N.Y.

Elgin, Jr., J. H. and Bauchan, G. R. 1986. Self-incompatibility—a mechanism for successfully producing hybrid alfalfa cultivars? Page 62 in Agronomy abstracts. American Society of Agronomy, Madison Wis.

He, Y. and Wu, R. 1987. A study of self-incompatibility in alfalfa. Chinese J. Prairie Sci. 4: 6–12.

Hill, R. R., Shenk, J. S. and Barnes, R. F. 1988. Breeding for yield and quality. Pages 809–825 in A. A. Hanson, D. K. Barnes, and R. R. Hill, eds. Alfalfa and alfalfa improvement. Agronomy no. 29. American Society of Agronomy, Madison, Wis.

Hunt, O. J., Barnes, D. K., Peaden, R. N. and Hartman, B. J. 1976. Inheritance of black seed character and its use in alfalfa improvement. Page 33 in Proc. 25th alfalfa improvement conf. Cornell Univ., Ithaca, N.Y. 13–15 July.

Lin, C. S. and Binns, M. R. 1988. A superiority measure of cultivar performance for cultivar × location data. Can. J. Plant Sci. 68: 193–198.

McKersie, B. D., Senaratna, T., Bowley, S. R., Brown, D. C. W. and Bewley, J. D. 1988. Progress toward the production of hybrid alfalfa using artificial seed technology. Page 48 in Proc. 31st. alfalfa improvement conf. Beltsville Ag. Res. Center, Beltsville Md. 19–23 June.

Melton, B. 1970. Effects of clones, generation of inbreeding and years on self-fertility in alfalfa. Crop Sci. 10: 497–500.

Mistinova, A. 1987. Intercompatibility of autoincompatible lucern clones. Vedecke Prace Vyskumneho Ustavu Rastlinnej Vyroby V Piest Anoch 21: 19–29.

Okazaki, K. and Hinata, K. 1987. Repressing the expression of self-incompatibility in crucifers by short-term high temperature treatment. Theor. Appl. Genet. 73: 496–500.

Pedersen, M. W. and Barnes, D. K. 1973. Alfalfa seed size as an indicator of hybridity. Crop. Sci. 13: 72–75.

Rumbaugh, M. D., Caddel, J. L. and Rowe, D. E. 1988. Breeding and quantitative genetics. Pages 777–808 in A. A. Hanson, D. K. Barnes, and R. R. Hill, eds. Alfalfa and alfalfa improvement. Agronomy no. 29. American Society of Agronomy, Madison, Wis.

Statistical Analysis System Institute. 1985. SAS user's guide: Statistics. SAS Institute, Inc. Cary, N.C.

Straley, C. and Melton, B. 1970. Effect of temperature on self-fertility and in vitro pollen growth characteristics of selected alfalfa clones. Crop Sci. 10: 326–329.

Stuart, D. A., Strickland, S. G. and Walker, K. A. 1987. Bioreactor production of alfalfa somatic embryos. Hortscience 22: 800–803.

Tysdal, H. M. and Kiesselbach, T. A. 1944. Hybrid alfalfa. Agron. J. 36: 649–667.

Viands, D. R., Sun, P. and Barnes, D. K. 1988. Pollination control mechanism: Mechanical and sterility. Pages 931–960 in A. A. Hanson, D. K. Barnes, and R. R. Hill, eds. Alfalfa and alfalfa improvement. Agronomy no. 29. American Society of Agronomy, Madison, Wis.